



MatriCal

High-Throughput Sonication for Various Biological Applications

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Abstract:

Sonication has historically been used for a variety of applications in the life science fields. Biological related applications include DNA shearing, cell poration and lysis, and tissue homogenization. Chemistry related applications include mixing, extraction, solubilization, and catalysis of chemical reactions. Traditional sonicators are either both sonicators, where the sonic energy is transferred from the transducer to the sample through water, or probe sonicators where a single metal probe inserted into the sample transfers the sonic energy. Both sonicators are limited by the amount of energy they can provide while probe sonicators have been limited in the number of samples that can be processed at any given time. A multi-probe sonicator would supply the means to perform the traditional applications that sonication has been used for all the while meeting the high-throughput demands of today's life science industry. The SonicMan™, a high-throughput sonication instrument, provides the means to utilize sonic energy for these applications in the high-throughput era.

The SonicMan™

The SonicMan is a high throughput multi-probe sonication instrument developed by MatriCal, Inc. configurable with 96, 384, and 1536 well formats (Fig 2A). The instrument is operated with a user-friendly touch screen interface. The SonicMan uses disposable gasketed pin lids (Fig 2B) to transfer sonic energy to each individual well and ensure no well to well cross contamination. Plates are placed on an extendable/retractable shuttle. The sonicator allows for power outputs between 100 and 1150 watts (12 watts/pin for 96 well formats and 3 watts/pin for 384 well formats at 100% power) and configurable time intervals from 1 to 20 seconds.

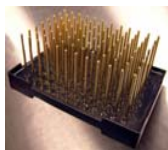


Figure 2A:
Multi-probe pinned lid.

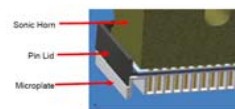


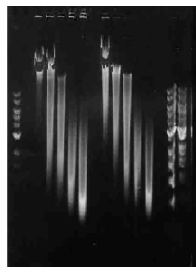
Figure 2b:
Diagram showing "sandwich" of sonic horn, pin lid, and microplate which contains samples.

- High-throughput sonicator compatible with 96, 384, and 1536 plate formats.
- Easy, rapid, configurable membrane disruption.
- Efficient macromolecule shearing.

Genomic DNA Shearing:

Small-insert random DNA fragments are necessary to create efficient libraries for a variety of biological applications. The success and efficiency of sequencing a large genome is dependent on the randomness of the fragments generated by the shearing of target DNA. Physical shearing methods (i.e., sonication, nebulization, and hydrodynamic shearing^{6,59}) are preferentially chosen over enzymatic digestion due to the randomness and size of the fragments produced resulting in a suitable overlapping collection of fragments for sub-cloning. The SonicMan offers these properties in a high-throughput sonication format allowing for a straightforward, user-friendly, customizable method to generate random DNA fragments. Fragment size is correlated with sonication settings (power/time) and is controllable by the user and fragments centered around a desired length can be repeatedly generated in seconds. Unlike enzyme based digestion, the random fragments generated by sonication are suitable for the sequencing of large genomes and sonication procedures allow for an uncomplicated, quick process as opposed to techniques like hydrodynamic shearing.

Figure 3: Shearing of Bacterial Genomic DNA



Lane	DNA	Sonication Time
1	1 kb Ladder	NA
2		10 Seconds
3		10 Seconds
4		20 Seconds
5		20 Seconds
6		40 Seconds
7		40 Seconds
8		60 Seconds
9		60 Seconds
10		10 Seconds
11		20 Seconds
12		40 Seconds
13		60 Seconds
14		60 Seconds
15	1 kb Ladder	NA
16	1 kb Ladder	NA

Bacterial DNA was sheared and a 70ul aliquot was transferred to a well in a 384 plate (MBB 101-1), MatriCal, Inc. Spokane, WA). The plate was sonicated in a SonicMan using a 384 disposable pinlid (SLO-384 p11, MatriCal, Inc. Spokane, WA) at 100% Power for times of 10, 20, 40, and 60 seconds.

After each sonication a 5ul aliquot was loaded into a Gel. The 7 agarose gels ran at 160 V for 70 min. Lane 1, 11, and 12 are 1kb DNA markers (NEB, Ipswich, MA).

Results:
As shown in figure 3, genomic DNA may be sheared to fragments with the distribution of sizes centered around the users preferred size. DNA may be sheared to generate fragments with a distribution of sizes centered around 10kb (lanes 4) or sheared to smaller fragments (4kb; lane 10, 20; lane 4, 20; lane 6). Fragments can be repeatedly produced with minimal production of unwanted "small fragments" (<300bp), as in lanes 4 & 10 of figure 1A, which can lower transformation efficiency. A simple configuration set is all that is required by a new user or with new DNA to correlate ideal settings to ideal fragment length.



Figure 1:
Front View of the SonicMan with touch screen panel

Membrane Disruption & Macromolecule Extraction:

A method to disrupt or lyse cells is invariably required in the research and production of intracellular products in the biochemical and biomedical industries. Requirements for cell lysis include speed (to avoid further biochemical changes), configurability (disruption power capable of lysing a range of membrane strengths, i.e., mammalian cells to yeast cells), and integration (ease of carryover from upstream applications and to downstream applications). Traditionally, cells have been lysed by non-mechanical methods, mechanical methods, or even using a combination of both. Non-mechanical methods such as detergents, alkali, and enzymatic degradation are typically selective and often require additional reagents and/or a removal process to continue on to downstream applications. Mechanical methods of cell disruption are often cumbersome, irreproducible, time-consuming, and prone to cross-contamination and as such are not suitable for high-throughput formats. Sonication has been a widely used, successful method for cell disruption⁶⁰ due to its speed, ease and cleanliness, and ability to lyse a range of cells but until now has only been relevant to single-sample techniques. The SonicMan continues the traditional method of cell lysis with sonication and extends it into the high-throughput format. The SonicMan offers the ability to lyse a variety of cells with configurable settings allowing for lysis of easily disrupted cells (i.e., insect or mammalian cells), to difficult to disrupt cells (i.e., E. coli cells), and to highly difficult to disrupt cells like yeast which is surrounded by a thick, tough, and rigid cell wall⁶¹.

E. coli Cell Lysis:

Bacteria Culturing

A 50 mL culture of *Escherichia coli* BL21 (DE) was grown in Luria-Bertani (LB) media overnight (16 hours) in 37°C water bath shaker. A fresh 250 mL LB culture was inoculated with 1 mL of these cells and grown for 4 hours to an OD₆₀₀ of between 400 and 700. The cells were collected with centrifugation (Beckman, 100g, 10min, 4°C), washed twice with chilled PBS Buffer, pH 7.5 and re-suspended in 50 mL of chilled PBS Buffer. Cell viability was determined after each sonication time (see sonication settings) by retrieving an aliquot and diluting appropriately for a serial dilution method.

Sonication Settings:

300 ul aliquots of re-suspended bacteria were transferred to 1.4 mL polyethylene tubes (Matrix, Hudson, NH) and the tubes placed in the corresponding 96-well-format Matrix tube rack. The plates were submerged to sonication times of 0, 10, 20, 30, 40, and 50 seconds at 100% power (watts/pin). The samples were then centrifuged (Galaxy 7, VWR, West Chester, PA) at 8000 rpm for 15 minutes to pellet debris. The supernatant containing solubilized proteins was collected for analysis.

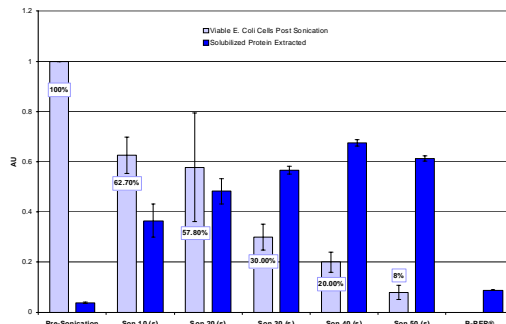
B-PeP Protocol:

300 ul aliquots of re-suspended bacteria were centrifuged (Galaxy 7, VWR, West Chester, PA) at 100g for 10 min, to pellet cells. The cells were re-suspended in 60 ul of B-PeP Reagent (Pierce, Rockford, IL) and vortexed for 1 minute corresponding to the B-PeP protocol. 20ul of B-PeP buffer was added to the solution (to normalize the solution with the sonicated samples) and the solution centrifuged to pellet debris and the supernatant collected for analysis.

Protein Assay:

The amount of protein released after each sonication time was qualitatively determined by use of Bradford Reagent (Sigma-Aldrich Chemical Company, St. Louis, MO). An aliquot of 100 ul of each sample was mixed with 900 ul of Bradford Reagent and the absorption at 595 nm recorded (Shimadzu UV-1601 UV-Visible spectrometer) after 10 minutes of mixing time.

Figure 4: E. coli Cell Lysis/Protein Extraction



Results:

The efficiency of cell disruption was quantified by the amount of soluble protein released determined by mixing with Bradford Reagent (Sigma-Aldrich, St. Louis, MO). The results indicate that at 100% sonication power the maximum protein release occurs at 40 seconds of sonication. As expected, protein release is inversely correlated with cells left intact. The SonicMan compared favorably with Pierce's B-PeP protein extraction reagent in their respective abilities to release proteins.

Yeast Cell Lysis:

Yeast Culturing:

A 50 mL O/N culture of *Saccharomyces cerevisiae* (Fleischmann's Bakers Yeast) was grown in Yeast extract Peptone Dextrose (YPD) broth overnight (16 hours) in a 30°C water bath shaker. Fresh YDP media (250 mL) was inoculated with 1 mL of the O/N culture and grown for 4 hours to an OD₆₀₀ of between 700-800 (log phase). The cells were collected with centrifugation (Beckman, 250g, 10min, 4°C), washed with chilled PBS Buffer, pH 7.5, and re-suspended in 50 mL of chilled PBS Buffer. Cell viability was determined after each sonication time (see sonication settings, below) by retrieving an aliquot and diluting appropriately for a serial dilution method.

Sonication Settings:

300 ul aliquots of re-suspended yeast cells were transferred to 1.4 mL polyethylene tubes (Matrix, Hudson, NH) and the tubes placed in their corresponding 96-well-format Matrix tube rack. The plates were submerged to sonication times of 0, 10, 20, 30 seconds at 100% power (12 watts/pin). The samples were then centrifuged (Galaxy 7, VWR, West Chester, PA) at 8000 rpm for 15 minutes to pellet debris. The supernatant containing solubilized proteins was collected for analysis.

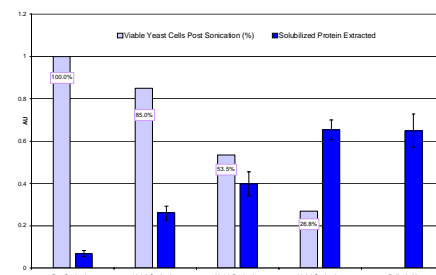
Cell Lysis Protocol:

300 ul aliquots of re-suspended yeast were centrifuged (Galaxy 7, VWR, West Chester, PA) at 250g for 10 min, to pellet cells. The cells were re-suspended in 20 ul of 5mlz pellet as recommended by Sigma protocol⁶² of Cell Lysis Yeast Cell Lysis Extraction Reagent (Sigma-Aldrich Chemical Company, St. Louis, MO) and gently shaken for 30 minutes corresponding to the Sigma protocol. 28ul of PBS buffer was added to the solution post mixing (to normalize the solution with the sonicated samples) and the solution centrifuged to pellet debris and the supernatant collected for analysis.

Protein Assay:

The amount of protein released after each sonication time and Cell Lysis-Y incubation was qualitatively determined by use of Bradford Reagent (Sigma-Aldrich Chemical Company, St. Louis, MO). An aliquot of 100 ul of each sample was mixed with 900 ul of Bradford Reagent and the absorption at 595 nm recorded (Shimadzu UV-1601 UV-Visible spectrometer) after 10 minutes of mixing time.

Figure 5: Yeast Cell Lysis/Protein Extraction



Results:

The efficiency of cell disruption was quantified by the amount of soluble protein released determined by mixing with Bradford Reagent (Sigma-Aldrich, St. Louis, MO). The results indicate that at 100% sonication power the maximum protein release occurs at 30 seconds of sonication. As expected, protein release is inversely correlated with cells left intact. The SonicMan results were comparable with Cell Lysis-Y Yeast Cell Lysis Extraction Reagent (Sigma-Aldrich, St. Louis, MO) in their respective abilities to release proteins.

Other SonicMan Biological Applications:

- Transfection of varying cell types with various macromolecules
- Tissue Homogenization
- Kinetic mixing of crowded biological solutions.
- Dissolution and solubilizing various biological significant items.
- Extraction of membrane bound proteins.

References:

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